ABSTRACT

HUNT, LINDA-MARGARET. An Autoradiographic Analysis of Mitotic Activity in Imaginal Wing Discs of *Drosophila virilis* *in Vitro*. (1971) Directed by: Dr. Edward McCrady III. pp. 39

One of the most extensively studied phenomena in developmental biology today is the transition of determined cells into differentiated cells. One of the techniques most often used in this area is tissue culture. Although this method of analysis allows the direct observation of cellular and tissue interactions, the results must be constantly interpreted in terms of their relation to *in situ* phenomena.

In the present study imaginal wing discs were excised from late third instar *Drosophila virilis* and cultured *in vitro* in Schneider's medium for zero to seven days. At the end of the culture period, the discs were labeled with tritiated thymidine for two and one half hours and sectioned for autoradiography. The developed autoradiographs were examined under light microscopy for evidence of uptake of label, which is directly correlated with the mitotic activity of the labeled cells.

A high rate of labeling was observed in the zero day discs. Statistically significant labeling was record-

ed in the discs cultured *in vitro* for one to four days. It was concluded that mitosis occurs in late third instar wing discs and that this mitotic activity rapidly declines during *in vitro* culture.

The developmental capacities of imaginal wing discs *in vitro*, *in vivo*, and *in situ* are discussed in terms of mitotic activities. It is suggested that the factor controling mitotic rate in all cases is juvenile hormone or a combined effect of juvenile hormone and ecdysone.

AN AUTORADIOGRAPHIC ANALYSIS OF MITOTIC ACTIVITY IN IMAGINAL WING DISCS OF *DROSOPHILA VIRILIS* IN VITRO

by

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A Thesis Submitted to
the Faculty of the Graduate School at
The University of North Carolina at Greensboro
in Partial Fulfillment
of the Requirements for the Degree
Master of Arts

Greensboro
May, 1971

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APPROVAL SHEET

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ACKNOWLEDGMENTS

I wish to thank Dr. Edward McCrady III whose excellent ideas and helpful suggestions have contributed so greatly to my understanding of biology and to the preparation of this thesis. To Dr. Laura Anderton and Dr. Bruce Eberhart I would like to extend my gratitude for their encouragement and constructive criticisms of this work.

I would also like to thank my many colleagues and friends at both the University of North Carolina at Greensboro and the University of Michigan, whose ideas of both a scientific and non-scientific nature have contributed greatly to my graduate career.

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INTRODUCTION

One of the most extensively studied phenomena in developmental biology today is the transition of determined cells into differentiated cells. Although the phenomenon has been observed for centuries, a disproportionately small amount of information is known about the mechanisms involved. Much of the experimental evidence amassed in this area in recent years has come from the application of new cytological techniques. One such technique, tissue culture, has been particularly useful. Tissue culture enables the experimenter to observe cellular and tissue interactions directly without the problems involved in maintaining whole organisms. However, the experimenter is constantly faced with the challenge of accurately relating his observations to *in situ* patterns of development.

The holometabolous insects have been widely used in experiments designed to study the mechanisms of determination and differentiation. They are uniquely suited for such studies because they possess two distinct tissue systems during their larval development. One system is determined embryonically and immediately differentiates into the functional larval structures. The second system is determined during embryogenesis, but does not

differentiate until pupation occurs. This system, the imaginal discs, forms the adult structures of the insect. In 1937, Ephrussi and Beadle perfected a microinjection technique whereby imaginal discs from the fruitfly, Drosophila, could be transplanted from one larva to another. After the host metamorphosed and the injected disc was recovered from its abdomen, Ephrussi and Beadle found that the injected tissue had also metamorphosed, forming its characteristic adult structures. In other words, the injected disc was determined and it subsequently differentiated simultaneously with its host.

Many experimenters were quick to take advantage of this demonstration of delayed differentiation. By imposing a period of culture time between removal of the discs and subsequent forced differentiation, it is possible experimentally to manipulate the imaginal discs and then directly observe the effects of these manipulations in the differentiated product.

Hadorn(1963, 1966) and his associates have worked extensively with in vivo culture of Drosophila imaginal discs. In this method, imaginal discs are removed from larvae and injected into the abdomens of adult flies. Such cells only metamorphose when they are subsequently transplanted into larvae. Other workers, including Schneider(1967), McCrady(1968, 1969), and Mandaron(1970),

have concentrated on *in vitro* culture. In this case, imaginal discs are cultured in a synthetic medium using techniques similar to those employed for vertebrate tissue culture. The effects on determination and differentiation of imaginal discs resulting from these two types of tissue culture are not the same. The present study is an attempt to obtain pertinent data necessary for the comparison of events observed *in vitro* and *in vivo* culture and wherever possible, to relate these to the *in situ* development of imaginal structures.

McCrady(1968) has found that if whole or half discs which have been cultured *in vitro* are subsequently transplanted into a larval host, the amount of differentiation that occurs in the transplanted tissue during metamorphosis depends in part on the length of culture time. Whole discs transplanted without culturing yield poor wing development and good thorax development. After seven days *in vitro* the wing area differentiates quite well, but the thorax develops very poorly. Three days *in vitro* before transplantation produces intermediate results. (For quantification of terms, see McCrady, 1968.)

In contrast to these *in vitro* results, Hadorn(1969) has been able to maintain whole wing discs and sections of discs *in vivo* culture for over seven years with no discernible change in their developmental capacities upon

forced differentiation. One extremely important change does occur, however. Imaginal cells cultured *in vivo*, proliferate very rapidly. Thus it is the descendants of the original disc cell population which retain the determination in extended culture.

Several possible hypotheses may be raised in explanation of these observations. The question is why do imaginal wing discs cultured *in vitro* show a change in developmental capacity over a seven day period, while similar cells cultured *in vivo* usually retain the normal capacity found *in situ*. Although many possible factors, such as differences in the media used and the transplanted tissue's possible interactions with the adult host, could be investigated, it was the mitotic activity of the discs which attracted this author's attention.

The extreme mitotic proliferation of discs *in vivo* (described above) has been well documented(Hadorn, *et. al.*, 1968; Wildermuth, 1968). Garcia-Bellido and Merriam(1971) used X-ray-induced somatic crossing-over to analyze the clonal development of wing discs of *Drosophila melanogaster in situ*. Their results indicate that the wing disc cells divide exponentially from the beginning of the larval period up to puparium formation. A total of 15.6 divisions is necessary to produce the 52,000 adult cells. The division rate appears to be constant in the intermolt

periods, but decreases during molting. An average cell cycle time of 8 hours 30 minutes was reported.

As yet no information has been available concerning the mitotic rates of late third instar imaginal discs *in vitro*. The experiments reported in this paper were designed to test the hypothesis that there is a definite correlation between the mitotic activity of imaginal discs when *in vitro* cultured and their subsequent developmental capacity. The objectives of the study were as follows: 1) To determine whether or not mitosis occurs in late third instar wing discs of *Drosophila virilis* and if so whether or not it continues *in vitro*; 2) If mitosis does occur, to determine if the rate of mitosis changes during the time in culture, ie., does the rate of mitosis of the cell population on the first day of culture differ from that on the second day; and, 3) If mitosis occurs, to study any pattern as to which cells in the population undergo mitosis and which do not.

In order to attain these objectives a technique is required which will facilitate the observation of mitosis at any given time. The technique most commonly used for vertebrate tissue is visual identification and counting of mitotic figures. Unfortunately, this is impossible in imaginal tissue because of the small size and number of chromosomes. The method used was autoradiography of iso-

topically labeled discs. This technique is based on the coupling of a physical phenomenon (the radioactive decay of a tritium molecule) with a specific biochemical reaction (the incorporation of thymine into DNA). After cellular uptake of the nucleoside, thymidine is converted to thymine which is one of the four bases of the DNA molecule. If tritium-labeled thymidine is supplied, it will eventually be bound in the nuclei of dividing cells in their DNA. Thus all cells which bind tritiated thymidine are undergoing DNA synthesis, a necessary preparation for mitosis. Based on these correlations, any system which records the amount of radioactivity present can be used as an assay for estimating the mitotic rate of labeled cells(Cleaver, 1967)

MATERIALS AND METHODS

Larvae of Drosophila virilis were used as the source of wing discs. All stages were raised on sterile David's medium(1962) at a constant temperature of 25 ± 0.25 degrees Centigrade.

Egg collections were made by inserting food trays into half-pint milk bottles containing thirty or more flies. The flies were allowed to deposit eggs for two hours, after which the trays were removed. The eggs were transferred by forceps to a dish containing a solution of 2% sodium hypochlorite. Immersion in this solution for ten minutes dechorionates the eggs(Poulson and Waterhouse, 1960). Dechorionation serves to rid the eggs of bacteria. The eggs were then transferred by sterile Pasteur pipette to sterile half-pint milk bottles containing David's medium. The organisms were then allowed to develop until they reached the late third instar (140-150 hours after hatching). This point is marked by the cessation of feeding, after which the larvae crawl out of the food and onto the sides of the container.

In preparation for dissection the larvae were removed from the bottles with sterile watch-maker's forceps and rinsed in sterile Waddington's insect saline.

After removal, the discs were washed in three changes of Schneider's medium(1964) which had been sterilized by GS Millipore filtration. Two 0.05ml drops of the medium, each containing one disc, were placed on the underside of a sterile coverslip which had been treated with a silicone solution (Beckman Desicote) to prevent spreading of the drop. The cover slip was mounted over the well of a depression slide which had been ringed with Vaseline in order to seal the culture.

Populations of from 20 to 35 discs were set up in the manner described and allowed to remain in culture for from one to seven days. The controls were freshly dissected discs which were never placed in culture.

After the appropriate time in culture, the discs were placed in 5ml of culture medium containing tritium-labeled thymidine. This radioactive medium was prepared by adding thymidine-methyl-H^3 (specific activity 6.7c/mM, New England Nuclear) to Schneider's medium, giving a final concentration of 5μc/ml. The discs were allowed to take up the label for two and one half hours. The time interval was based on experiments done by Robb(1969) and confirmed by a series of trial autoradiographs.[1]

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[1]There is no rule of thumb by which the time required for sufficient uptake of radioisotopes can be calculated. As a starting point for determining an effective

The labeled discs were then rinsed in three changes of non-labeled Schneider's medium to remove any label adhering to their surfaces and fixed in Carnoy's fixative overnight.

The discs were dehydrated, stained with 0.5% Toluidine blue, and embedded in paraffin.

Application of stripping film (Kodak AR-10) was carried out in a darkroom at a distance of three feet from a safe-light containing a deep red filter and a 15 watt bulb. Pieces of film 1 X 2½ inches in size were cut from the emulsion sheets using a single edged razor blade. The film was floated, emulsion side down, on the surface of a dish of water. After allowing ½ to 1 minute for expansion of the film, the slide was lowered beneath the floating strip, raised, and wrapped in the film.

The slides were air-dried and sealed in light-tight slide boxes containing a drying agent. Exposure time was five weeks.

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culture period, a literature survey was conducted to investigate the intervals used by other experimenters. The time chosen, two and one half hours, was based on experiments done by Robb in 1969. Although Robb's assay technique was liquid scintillation counting (see discussion, page 28) rather than autoradiography, he was able to demonstrate label uptake in wing discs of Drosophila melanogaster cultured in vitro, using this incubation time.

The developed slides were examined under bright field illumination at 40X magnification and random grain counts were made. The following sampling procedure was used. Five sections of tissue on each slide were chosen at random. Five grain counts were then made over each of these sections. Each grain count was made within an area of $585\mu^2$, delimited by use of a standard ocular micrometer. A mean was calculated for the counts/$585\mu^2$ for each tissue section. The means from each population of discs, ie., all one day discs, all two day discs, etc., were then pooled using an Olivetti Underwood Programma 101 computer. The population means, standard deviations, and standard errors of the means were calculated and compared using the Student's t test.

The mean grain count/unit area is the criterion used in this paper for comparing mitotic rates.

RESULTS

Table I and Figure 1 summarize all the results obtained.

Presence of Mitotic Activity in Late Third Instar Wing Discs

The very heavy uptake of label in the zero day discs indicates that extensive mitotic activity is occurring in late third instar wing discs. The grain distribution also indicates that the cell divisions are occurring uniformly in all areas of the discs. (See Figure 2.) No regional specificity was observed.

Correlation of the Length of Time *in Vitro* and the Rate of Mitosis

The mean grain count/unit area for the zero day discs was 40.27± 10.75. After one day in culture the count dropped to 16.09± 5.36 and remained at this level through the second day (16.22± 3.22). Each succeeding day the count continued to decrease. There is no significant difference among the counts from the fifth, sixth, and seventh day, nor between them and the background count. Thus it is concluded that mitosis does continue *in vitro*, at a reduced rate and for a short time, after which all mitosis stops.

Regional Patterns of Mitotic Activity

The slides were carefully examined in an attempt to detect any regional specificity of mitosis within a disc. One such trend was observed. In the vast majority of the sections examined, the mitotic activity of the sections was restricted to the internal area of the discs. Peripheral counts were extremely low. No other regional specificity was observed. In all cases the thorax area count was similar to the wing area count.

Table I: Statistical Analysis of Data from Grain Counts Made from Autoradiographs.

Number of Days *in Vitro*	Number of Sections Counted	Mean Counts/ Unit Area	Standard Deviation	Standard Error
0	14	40.27	10.75	2.87
1	15	16.09	5.36	1.38
2	15	16.23	3.22	0.86
3	40	8.81	2.52	0.40
4	20	6.64	1.74	0.39
5	11	4.58	1.47	0.44
6	35	4.23	1.35	0.23
7	20	3.70	0.82	0.18
Background	40	3.34	1.38	0.22

Figure 1: Mean Grain Counts per Unit Area. Points represent the means. Bars indicate the standard deviations.

Figure 1

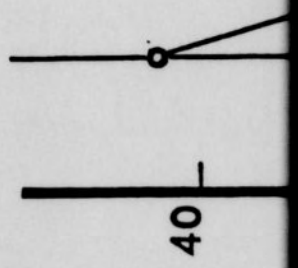


Figure 1

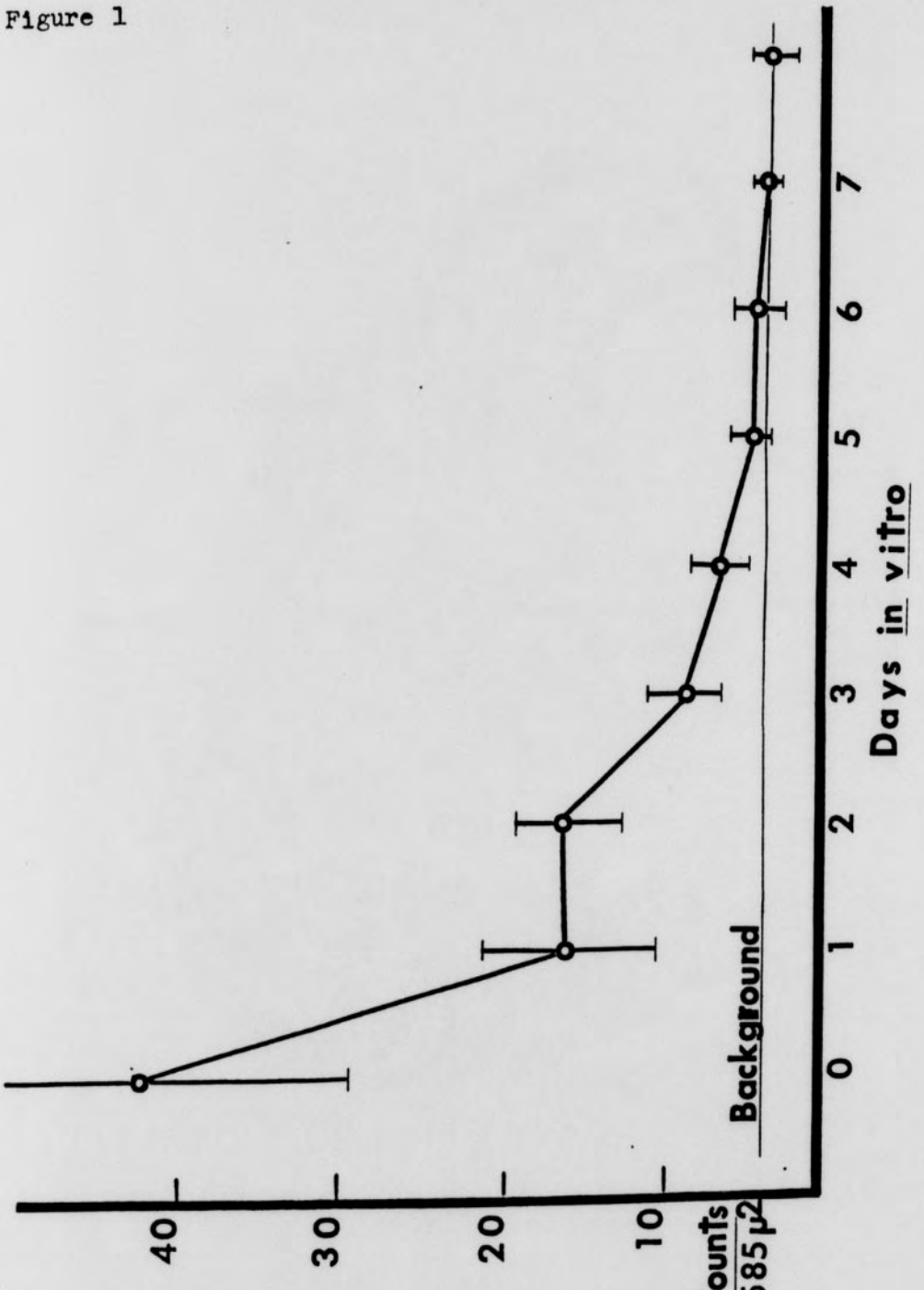


Figure 2: Autoradiograph of a Section of a Zero Day Disc.

Figure 2

Figure 3

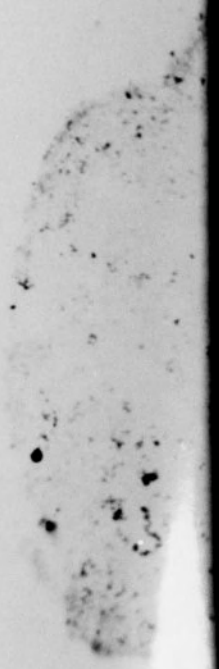


Figure 3: Autoradiograph of a Section of a Disc *in Vitro* for Two Days.

Figure 3

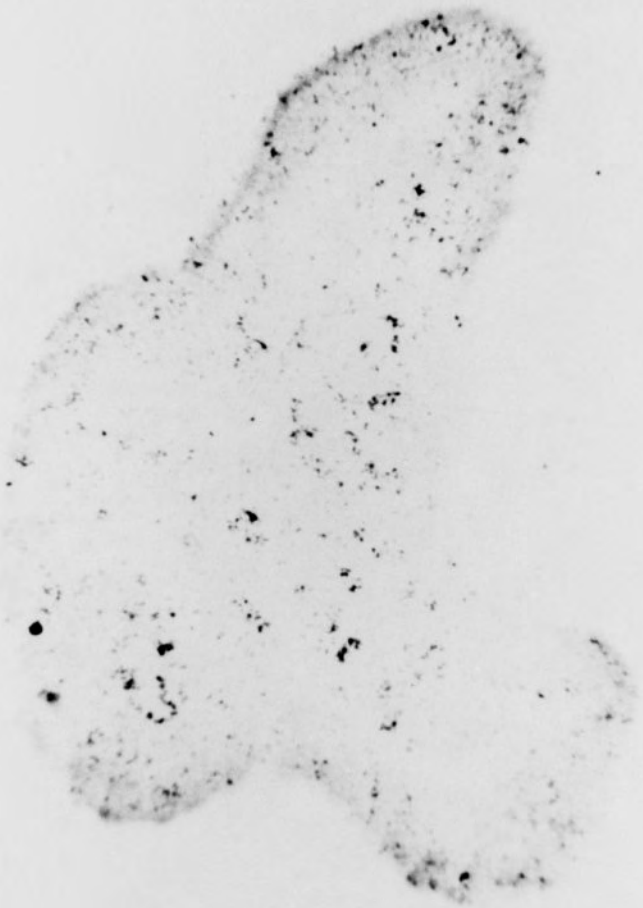
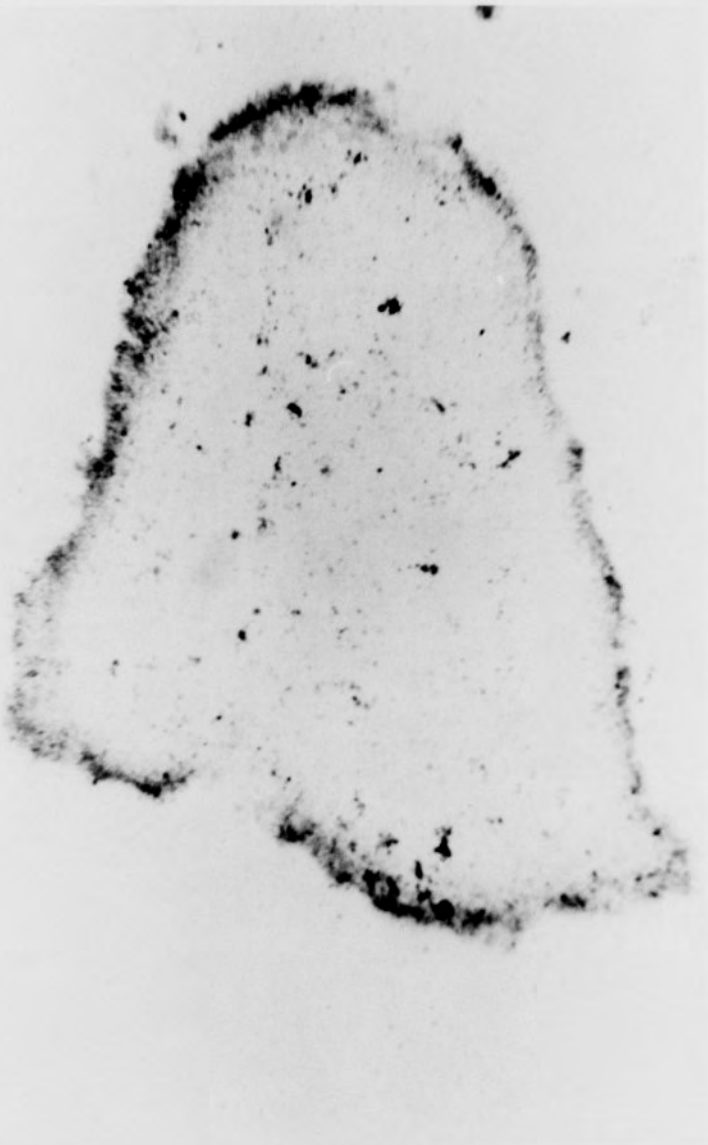


Figure 4

Figure 4: Autoradiograph of a Section of a Disc *in Vitro* for Four Days.

Figure 4



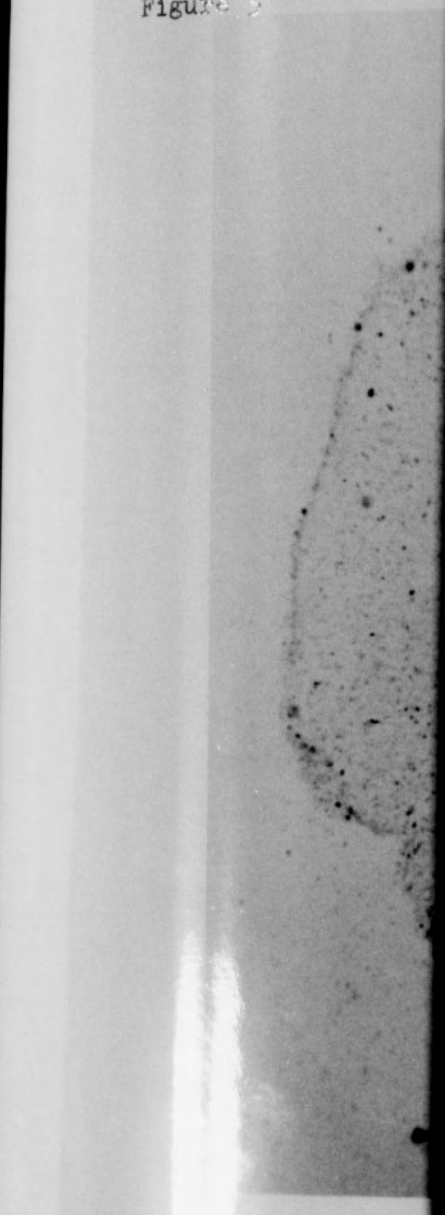
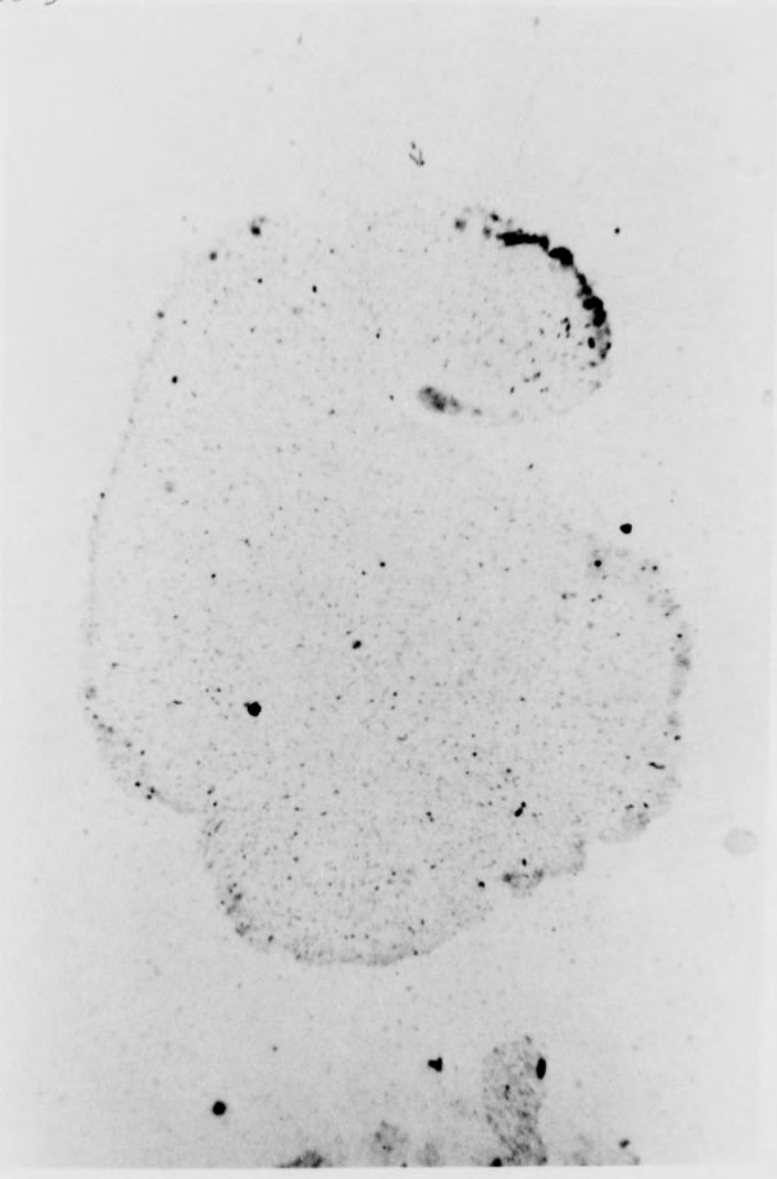


Figure 5: Autoradiograph of a Section of a Disc *in Vitro* for Seven Days.

Figure 5



DISCUSSION

Since 1960 when *in vitro* culture of insect tissue was sufficiently refined to be successfully applied to problems of development, many researchers have used the technique. Schneider(1967) reported that the cells of imaginal tissue so cultured do not appear to divide (or do so at a very low rate). This question of cell division *in vitro* was the subject of this study.

In 1908 when a frog which had been placed in a solution containing radium was removed and covered by a photographic plate, a new tool for biological research was discovered(Gude, 1968). The technique, called autoradiography, is based on the same principle as photography. Beta or alpha particles emitted by decomposing radioactive atoms produce an image on a photographic emulsion. The emulsion is a suspension of crystals of a silver halide (usually silver bromide), embedded in gelatin. When crystals of silver bromide are struck by beta particles, the silver atoms are ionized and form a latent image. After the emulsion is developed and fixed, each aggregate of reduced silver atoms becomes visible as a black dot on the emulsion. The distribution and combination of the dots make up the photographic image. In autoradiography, it is

satisfactory to look at the negative image since the clustering of developed ions, appearing under light microscopy, supplies all the information needed.

Today autoradiography is precise enough to locate radioactively labeled substances in individual cells and cell parts. Two conditions must be met to achieve this high resolution: 1)the radiation from the radioactive element must be of very short range, and 2)the cells must remain in close contact with the emulsion throughout the various experimental manipulations.

Shortness of range is satisfied by use of tritium, since its beta particles travel only about 1 micron in tissue. This is because its betas have a particularly low energy (0.02Mev). This minimizes the amount of their scatter and ionization effects are maximal very close to the emitter. This atom is thus the most effective in ionization of halide atoms in a monolayer emulsion on top of an ultra thin section of tissue. The condition of close contact between cells and emulsion is achieved by both liquid emulsions and stripping films.

After the film is applied by either technique, it is allowed to expose while stored in a light-tight box. During this period disintergrating radioactive atoms within the cells continue to emit beta particles, which in turn produce the latent image. The fixing process removes all

un-ionized atoms so that the emulsion is reduced to a thin, transparent film containing only the clusters of developed dots.

Presence of Mitotic Activity in Imaginal Discs

Many researchers have used autoradiography to determine the rate of DNA synthesis in *Drosophila* tissue. This is done by using a DNA precursor such as thymidine as the radioactive substance. Studies have been done using freshly excised salivary glands by Perkowska(1963), Howard and Plaut(1968), and Doyle and Laufer(1969). Autoradiographic studies of freshly excised imaginal discs have been reported by Krishnakumaran, *et. al.*(1965) working with the moth *Samia cynthia ricini*. They found that constant DNA synthesis occurred *in situ* during the eight days immediately preceding the larval-pupal molt. The purpose of their study was to investigate the *in situ* effects of ecdysone, the molting hormone. Therefore, no *in vitro* culturing was attempted. Their conclusions do, however, have bearing on the current study. They have postulated that it is ecdysone which stimulates DNA synthesis in the larval stages of insect development and that the imaginal discs' threshold is low enough to be constantly stimulated *in situ*.

In 1968 Wildermuth used similar techniques to study DNA synthesis in the labial discs of *Drosophila melanogaster* during *in vivo* culture. His results indicate mitosis

is occurring in cells scattered at random in the late third instar discs after two or three hours in culture. He confirms the conclusion of Hadorn, et. al.(1968) that cell division continues at a rapid rate throughout the in vivo culture period.

In the present study it was found that late third instar wing discs of Drosophila virilis were undergoing rapid cell division at the time of their removal from the larvae and that division continued in vitro. It might be suggested that the removal itself stimulated the initial rapid division, but other experimental data contradict this conclusion. First, recent studies by Garcia-Bellido and Merriam(1971) indicate that rapid cell division continues in situ in the wing discs of Drosophila melanogaster until twenty-four hours after puparium formation. Second, the labeling was quite heavy and uniformly distributed throughout each disc. If shock caused an increase in mitotic activity, the thorax area of the disc would be expected to have the highest mitotic rate since it is the only part of the disc in direct contact with the larval hypoderm. This assumption is supported by the observation of Hadorn and Buck(1962) that the edges of cut wing discs produce abnormally high numbers of bristles, presumably due to localized mitosis at the site of the cut. No such areas were observed in the present study.

Correlation of the Length of Time *in Vitro* and the Rate of Mitosis

Although *in situ* and *in vivo* studies have been done (Garcia-Bellido and Merriam, 1971 and Wildermuth, 1968, respectively), much less work has been done on *in vitro* DNA studies. Robb's 1969 paper deals with DNA synthesis in imaginal discs of *Drosophila melanogaster*. The purpose of that investigation was to establish the utility of a new chemically defined medium for insect tissue culture. The maintenance of DNA synthesis over a twenty-four hour period was presented as evidence of the medium's adequacy.

As the data in Table I indicate, labeling was observed after *in vitro* culture for as long as four days in the current study. The greater length of time may reflect the superiority of Schneider's medium in supporting cell division or a difference in the tolerances of *D. virilis* and *D. melanogaster* to tissue culture. No direct comparison of results can be made because Robb used scintillation counting rather than autoradiography for determining uptake of label. Although both of these processes record the occurence of radioactive disintergrations, they employ very different detectors. While autoradiography relies on the ability of radiation to ionize silver grains, scintillation counting makes use of the light emitted when certain chemicals are struck by high energy electrons. These

flashes of light or scintillations are recorded in a photomultiplier tube and registered as counts per minute. Of the two methods, liquid scintillation counting is far more accurate in determining total activity present, but it yields no information relative to location and number of cells dividing. One other major difference in technique should be mentioned. In Robb's work the discs were cultured exclusively in labeled medium rather than being placed in it for a two and one-half hour pulse label period. This extensive exposure to radioactive thymidine may have caused chromosome damage, which would account for the cessation of mitosis after only twenty four hours.

The observed rate of mitosis does not suggest that in vitro culture stimulates mitotic activity, nor does it appear to be adequate to maintain it. The longer in culture, the lower the rate dropped until it became completely undetectable.

Regional Patterns of Mitotic Activity during in Vitro Culture

In light of the hypothesis stated earlier that there is a definite correlation between the regional mitotic activity of the wing disc and its differential capacities, the sections were examined for evidence of such area specificity.

The anticipated decrease in mitotic activity of the thorax area of the discs was observed. The data cited here favorably agree with McCrady's(1968) results. In the zero day discs mitosis was high. At the end of the third day the rate had dropped to approximately one fifth of the original thorax rate and after seven days in vitro, mitosis had ceased.

However, the hypothesis as stated loses all value when the mitotic activity of the wing end of the discs is examined. It is found to correspond exactly to that of the thorax area. (See Figures 2, 3, 4, and 5.) Yet McCrady's results(1968) conclusively show that the cells of the wing area improve in their differential capacity as culture time increases.

The examination of other experiments by McCrady(1968) and those of Mandaron(1970) suggests another possible explanation of the results. McCrady's in vitro experiments using wing discs cut into separate thorax and wing areas indicate that the thorax in some way modifies the development of the wing. Wing areas cultured alone or in the same drop with a thorax area show intermediate development, regardless of the length of time in culture. Thorax sections alone in culture, do well on the first day, intermediately on the third day and very poorly after seven days in culture. Mandaron's whole disc experiments confirm these

results and indicate that *in vitro* culture in no way effects the determination of the discs.

If Mandaron's conclusions are correct, the phenomena observed by McCrady must have a physiological rather than genetic explanation. One possible explanation will be discussed below. When the discs were examined in order to detect any regional distributions of labeling, one such pattern was observed. A peripheral band of non-dividing cells was found around all of the sections cultured for twenty four hours or more. The width of the band did not increase with the time in culture. Thus the band may have been serving as a barrier prohibiting the influx of the substance which blocked the mitotic activity of the band or prohibiting any further rapid efflux of some substance required for mitotic activity. If either were the case, the mitotic activity of the central cells would be expected to decrease uniformly, which it does.

The possibility must also be considered that the barrier may have blocked the influx of tritiated thymidine. Wildermuth's experiments(1968) show that imaginal cells are highly permeable to tritiated thymidine after more than a week *in vivo*. Since results from *in vivo* and *in vitro* studies are not strictly comparable, no definite conclusion can be drawn on this point at present.

Table II: A Qualitative Summary of Pertinent *in Situ*, *in Vivo*, and *in Vitro* Developmental Characteristics.

Culture	Time	Mitotic Rate	Ecdysone	Juvenile Hormone	Developmental Capacity
In Situ	late third instar	moderate	high	low	normal
In Vivo	third day	very high	very low	high	normal or transdetermination[1]
In Vitro	third day	very low	very low	very low	poor

[1]Hadorn and his associates have observed that *in vivo* culture promotes the rapid cell division of imaginal tissue, but does not allow differentiation to take place while in culture. Subsequent forced differentiation can be achieved by transplanting the cultured tissue into larval hosts. After metamorphosis, Hadorn has found the original determination is maintained in most cases, but may be totally absent in others. These changes in determination, called transdeterminations, have been directly related to the increased cell divisions occurring during *in vivo* culture(Nothiger and Schubiger, 1966 and Garcia-Bellido, 1966). No transdeterminations have been observed *in vitro* to date. The results of the current study indicate, however, that such observations would not necessarily refute the Hadorn hypothesis of transdetermination based on increased cell division. Cell division does occur *in vitro*. Therefore, if the Hadorn hypothesis is correct, transdetermination is theoretically possible *in vitro*, but would probably require much more time in culture.

Comparison of *in Vitro*, *in Vivo*, and *in Situ* Mitotic Activities

The information listed in Table II was compiled from the works of Wildermuth(1968), Krishnakumaran, *et. al.* (1965), Garcia-Bellido and Merriam(1971) and Hadorn(1969) and from the current study. Although there are thousands of points for comparison among the three situations, the primary effectors of mitotic rates in each case appear to be identical. These effectors are ecdysone and juvenile hormone. As outlined earlier, Krishnakumaran, *et. al.* have postulated a role for ecdysone as the regulator of DNA synthesis in most insects. Contrary to their findings, the data above imply that the mitotic rate varies more directly with the concentration of juvenile hormone, while the ecdysone concentration effects the developmental capacity. Although the exact mechanisms are still unclear, a synergistic effect is likely.

A plausible explanation of the differences in developmental accomplishment in thorax and wing areas after *in vitro* culture can be presented on the basis of the foregoing information. It is possible that the thorax and wing areas have different thresholds of ecdysone and juvenile hormone required for the maintenance of life. Since neither of these hormones are present in Schneider's medium, the peripheral barrier could aid in conservation of

the hormones within the disc.

Although this hypothesis explains, at least in a heuristic way, the decline of the mitotic index of the thorax, it does not account for the retarded development of the wing in zero day discs. One possibility is that the lack of an intact tracheal oxygen suppy may more severely effect the wing area due to its distal position relative to the tracheal system. The longer the culture time, the greater the number of thorax cells which die or possibly the higher the threshold of hormone level required to stimulate their differentiation. In such a scheme the wing area would have a lower hormone threshold required for maintenance and would have therefore retained its ability to differentiate normally. The lack of competition from the thorax after its loss of activity on the fourth day in culture would permit the acquisition of needed oxygen while leaving the differential capacity of the wing area basically uneffected.

At best these complicated hypotheses indicate the extreme complexity of the questions of development even in this rather isolated situation. They are subject, however, to experimental testing. Further autoradiographic studies using radioactive precursors of RNA and protein synthesis and labeled nutrients would effectively show the validity of the barrier hypothesis. <u>In vitro</u> studies using hormone-

supplemented medium should provide the evidence needed to clarify the threshold concept as set forth here. If such experiments can succeed in maintaining DNA synthesis, the next logical step, the *in vitro* metamorphosis of imaginal discs, should not be too far in the future.

SUMMARY

1. Imaginal wing discs were excised from late third instar (140-150 hours after hatching) Drosophila virilis and cultured in vitro in Schneider's medium for zero to seven days.

2. At the end of the culture period the discs were labeled with tritiated thymidine for two and one half hours and sectioned for autoradiography.

3. The developed autoradiographs were examined for evidence of uptake of label, which is directly correlated with the mitotic activity of the labeled cells.

4. A high rate of labeling was observed in the zero day discs(controls). Statistically significant labeling was recorded in the discs cultured in vitro for one to four days.

5. It was concluded that mitosis occurs in late third instar wing discs and that this mitotic activity rapidly declines during in vitro culture.

6. The developmental capacities of imaginal wing discs in vitro, in situ, and in vivo are discussed in terms of mitotic activities. It is suggested that the factor controling mitotic rate in all cases is juvenile hormone or a combined effect of juvenile hormone and ecdysone.

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